Rapid Genome-wide Aneuploidy Diagnosis Using Uncultured Amniocytes and Array Comparative Genomic Hybridization in Pregnancy With Abnormal Ultrasound Findings Detected in Late Second and Third Trimesters

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A 32-year-old, gravida 2, para 1, woman was referred to the hospital at 23 weeks of gestation because of fetal cardiac abnormalities detected by sonography. The woman and her husband were non-consanguineous and healthy, and there was no family history of congenital heart defects. The woman had a cousin who suffered from mental retardation and Down syndrome. Level II ultrasound revealed a singleton fetus with a fetal biometry equivalent to 23 weeks of gestation and a ventricular septal defect. Amniocentesis was performed, and 36 mL of amniotic fluid was aspirated. About 20 mL of amniotic fluid was used for array comparative genomic hybridization (aCGH) using uncultured amniocytes, and 15 mL was used for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, bacterial artificial chromosome (BAC)-based aCGH showed trisomy 21 [arr cgh 21p11.2q22.3 (RP11-430M17 → RP11-1000I21)×3] (Figure 1A). Fluorescence *in situ* hybridization (FISH) analysis of the cultured interphase amniocytes using a combination of BAC probes RP11-135B17 (21q22.3) (47,931,911-48,108,188) (spectrum green) and RP11-450H16 (13q34) (114,940,140-115,099,262) (spectrum red) showed three green

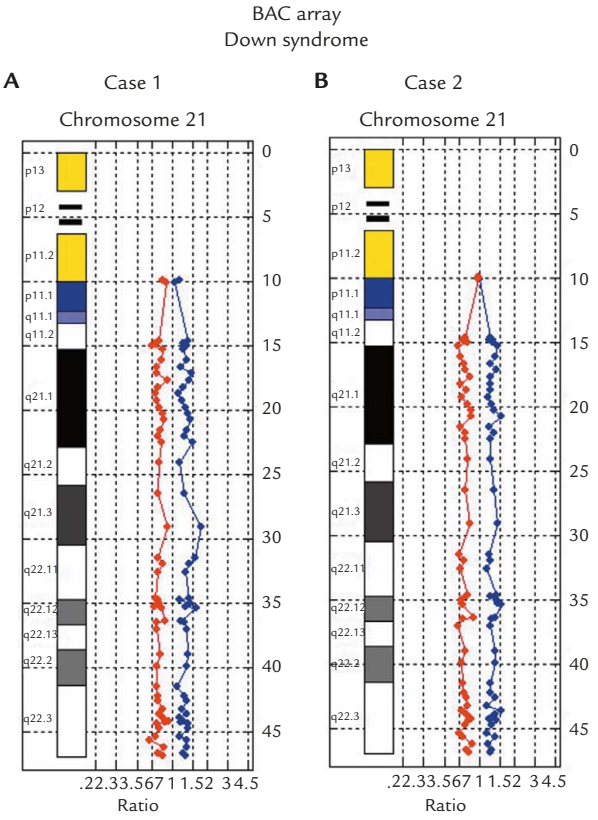

Figure 1. Bacterial artificial chromosome (BAC)-based array comparative genomic hybridization analyses using CMDX (CMDX, Irvine, CA, USA) BAC array comparative genomic hybridization CA3000 chips. (A) Case 1 and (B) Case 2 showed duplications of chromosome 21 consistent with the diagnosis of trisomy 21.

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signals and two red signals, consistent with the diagnosis of trisomy 21 (Figure 2A). Eight days after amniocentesis, conventional cytogenetic analysis revealed a 47,XX,+21 karyotype. Polymorphic DNA marker analysis using quantitative fluorescence polymerase chain reaction (QF-PCR) showed that the trisomy 21 in this fetus was likely to have been caused by paternal meiosis II nondisjunction or postzygotic mitotic nondisjunction (Figure 3).

A 33-year-old, gravida 2, para 1, woman was referred to the hospital at 30 weeks of gestation because of fetal brain abnormalities detected by sonography. The woman and her husband were non-consanguineous and healthy, and there was no family history of congenital anomalies. Level II ultrasound revealed a singleton fetus with a fetal biometry equivalent to 31 weeks of gestation and bilateral ventriculomegaly with a right ventricular width of 1.7 cm and a left ventricular width of 1.3 cm. Amniocentesis was performed, and 40 mL of amniotic fluid was aspirated. About 20 mL of amniotic fluid was used for aCGH using uncultured amniocytes, and 20 mL was used for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, BAC-based aCGH showed trisomy 21 [arr cgh 21p11.2q22.3 (RP11-1144F24 → RP11-1000I21) × 3] (Figure 1B). FISH (Figure 2B) and QF-PCR (Figure 4) analyses using cultured amniocytes revealed results consistent with trisomy 21. Thirteen days after amniocentesis, conventional cytogenetic analysis revealed a 47,XX,+21 karyotype. The parental DNA was not available. According to polymorphic DNA marker analysis, trisomy 21 in this fetus was likely to have been caused by meiosis I nondisjunction.

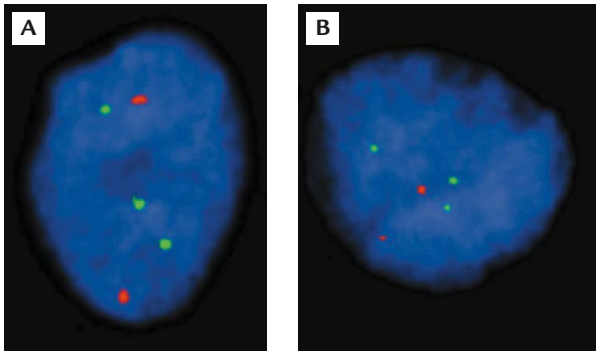

Figure 2. Fluorescence *in situ* hybridization analyses of interphase amniocytes using bacterial artificial chromosome probes RP11-135B17 (21q22.3) (spectrum green) and RP11-450H16 (13q34) (spectrum red). (A) Case 1 and (B) Case 2 showed three green signals and two red signals, consistent with the diagnosis of trisomy 21.

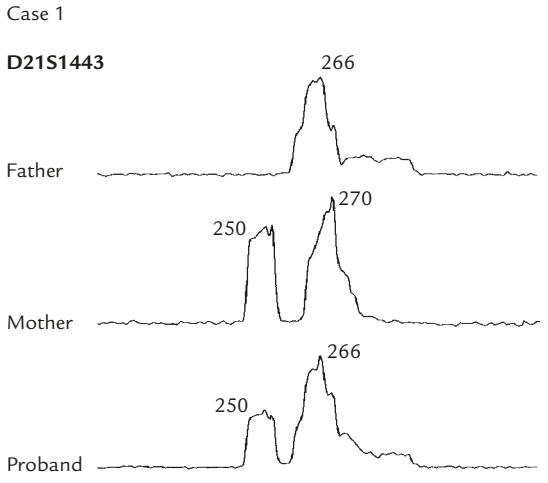

Figure 3. Representative electrophoretogram of quantitative fluorescence polymerase chain reaction assays in Case 1 at short tandem repeat markers for chromosome 21q. Two peaks (250 bp and 266 bp) of unequal fluorescence activity from different parental alleles (maternal:paternal) at a ratio of 1:2 were detected in the cultured fetal tissues using the marker D21S1443. This indicated a homologous duplication of chromosome 21q of paternal origin and was likely to be the result of meiosis II nondisjunction or postzygotic mitotic nondisjunction.

We have previously described the prenatal diagnosis of aneuploidy by aCGH using cultured or uncultured amniocytes [1,2]. The current cases further demonstrated that amniocentesis for genome-wide analysis using uncultured amniocytes and aCGH is a useful alternative to cordocentesis for rapid aneuploidy diagnosis in pregnancies with abnormal ultrasound findings detected during the late second and third trimesters.

Amniocentesis is simpler and less invasive than cordocentesis. The fetal loss rate is 0.5% for amniocentesis [3,4], compared with 0.8–2.5% for cordocentesis [5–8]. Cordocentesis is an invasive procedure associated with risks such as fetal loss, fetal bleeding and hemorrhage, fetal bradycardia, fetomaternal transfusion, umbilical cord thrombosis and hemorrhage,

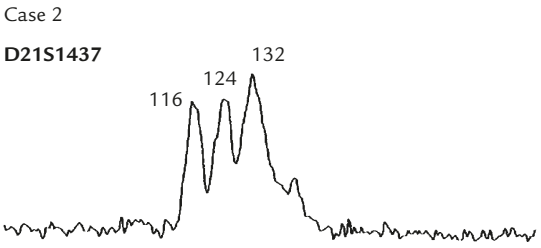

Figure 4. Representative electrophoretogram of quantitative fluorescence polymerase chain reaction assays of Case 2 at short tandem repeat markers for chromosome 21q. Three peaks (116 bp, 124 bp and 132 bp) of equal fluorescence activity at a ratio of 1:1:1 were detected in the cultured amniocytes. This indicated a heterologous duplication of chromosome 21q and was likely to be the result of meiosis I nondisjunction. The parental DNA was not available.

infection, abruptio placentae, and premature labor, even when performed in expert centers [9]. However, cordocentesis is preferable to amniocentesis for conventional cytogenetic analysis, especially in the late second and third trimesters, because it requires less time for cell culture. Fetal karyotypes can be obtained by culture of fetal cord blood lymphocytes from cordocentesis in 2–4 days. However, rapid karyotyping by cordocentesis has been replaced by amniocentesis combined with FISH or QF-PCR [10–15]. FISH and QF-PCR results can be obtained in 24–48 hours, but can detect only specific aneuploidies according to the specific probes or primers used. Recently, aCGH using cultured or uncultured amniocytes has been successfully applied for the prenatal diagnosis of chromosome abnormalities [16–27]. aCGH can detect unbalanced structural and numerical chromosome abnormalities of less than 100 kb, whereas conventional cytogenetic analysis can detect only microscopically visible deletions or duplications of 5–6 Mb at the 500-band level [25]. Tyreman et al [26] found that high-resolution array testing would benefit at least 10% of obstetric patients with abnormal ultrasound findings and a normal karyotype result. aCGH has the advantage of providing a rapid genome-wide study without the need for cell culture. However, aCGH also has the disadvantage of being unable to detect balanced translocations, inversions or polyploidy. Detection of low-level mosaicism depends on the dynamic range of the array used, and several studies have shown that aCGH can detect low-level mosaicism not revealed by conventional cytogenetics [25,28–30]. We, therefore, suggest that in a addition to rapid karyotyping by aCGH, conventional cytogenetic analysis is still required to rule out the presence of balanced translocations, inversions and polyploidy.

Acknowledgments

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